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Glucose promotes membrane cholesterol crystalline domain formation by lipid peroxidation

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ABSTRACT

Oxidative damage to vascular cell membrane phospholipids causes physicochemical changes in membrane structure and lipid organization, contributing to atherogenesis. Oxidative stress combined with hyperglycemia has been shown to further increase the risk of vascular and metabolic diseases. In this study, the effects of glucose on oxidative stress-induced cholesterol domain formation were tested in model membranes containing polyunsaturated fatty acids and physiologic levels of cholesterol. Membrane structural changes, including cholesterol domain formation, were characterized by small angle X-ray scattering (SAXS) analysis and correlated with spectrophotometrically-determined lipid hydroperoxide levels. Glucose treatment resulted in a concentration-dependent increase in lipid hydroperoxide formation, which correlated with the formation of highly-ordered cholesterol crystalline domains (unit cell periodicity of 34 Å) as well as a decrease in overall membrane bilayer width. The effect of glucose on lipid peroxidation was further enhanced by increased levels of cholesterol. Treatment with free radical-scavenging agents inhibited the biochemical and structural effects of glucose, even at elevated cholesterol levels. These data demonstrate that glucose promotes changes in membrane organization, including cholesterol crystal formation, through lipid peroxidation.

1. Introduction

Oxidative modification of membrane lipids contributes to the pathophysiology of cardiovascular disease and associated risk factors such as diabetes, dyslipidemia and hypertension [1,2]. Insulin resistance and poor glycemic control are directly associated with mechanisms of atherosclerosis by various chemical pathways, including non-enzymatic glucose reactions [3,4]. Several studies of glucose autoxidation focus primarily on the biochemical effects of the glycation products generated from the Maillard reaction of glucose with amine groups on proteins and lipids [5,6]. Although the biochemical and physiological effects of these glycation products have been studied extensively [7,8], there has been less attention paid to the biophysical effects of glucose-induced oxidative damage to non-amine membrane lipids [9,10]. The effect of glucose on polyunsaturated fatty acids (PUFA) and cholesterol is especially important since alterations of these lipids have been shown to promote vascular disease and insulin resistance [11,12]. Oxidation of low-density lipoproteins (LDL), in particular, contributes to atherosclerotic foam cell formation in the arterial wall [13]. In addition, peroxidation of plasma membrane lipids leads to the formation of cholesterol crystalline domains in vascular smooth muscle cells, which can cause inflammation and plaque instability [14,15].

In this study, we used small angle X-ray scattering (SAXS) to examine the physicochemical effects of glucose on membrane lipid peroxidation and structural organization in vesicles composed of fixed levels of PUFAs and cholesterol without adding exogenous radical initiators. Pronounced changes in membrane lipid organization were observed with glucose treatment, including the formation of discrete cholesterol crystalline domains and a reduction in overall membrane width. Concurrent spectrophotometric measurements showed a correlation between these physical changes and an increase in lipid hydroperoxide (LOOH) formation (an intermediate product of oxidative lipid damage) that could be inhibited with lipophilic agents that have antioxidant scavenger properties. However, when compared to glucose treatment, membrane treatment with the structurally similar sugar alcohol, mannitol, resulted in significantly less LOOH formation.

2. Materials and methods

2.1. Materials

1,2-Dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC) and 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine (DAPC) dissolved in

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HPLC grade chloroform were purchased from Avanti Polar Lipids (Alabaster, AL) in sealed ampules and stored at −80 °C. Powder cholesterol was also purchased from Avanti Polar Lipids and the prepared stock solution in HPLC grade chloroform was stored at −20 °C. Atorvastatin *o*-hydroxy metabolite (ATM) and amlodipine besylate (AML) were provided by Pfizer, Inc (Groton, CT) and 500 μM stock solutions were prepared in ethanol. Other chemicals were purchased from VWR (West Chester, PA) or Sigma-Aldrich (Saint Louis, MO). 100 mM glucose and mannitol solutions were prepared in diffraction buffer (0.5 mM HEPES, 154 mM NaCl, pH 7.3) and diluted with additional buffer to achieve the desired sample concentrations. CHOD-iodide colorimetric reagent was prepared, with modifications, from El-Saadani's method [16] and consisted of 200 mM K_2HPO_4, 120 mM KI, 0.15 mM NaN_3, 0.01 mM ammonium molybdate and 0.278 mM benzalkonium chloride. At the beginning of each experiment, 24 μM disodium ethylenediaminetetraacetic acid (EDTA) and 20 μM butylated hydroxytoluene (BHT) were added as antioxidants to an aliquot of CHOD-iodide reagent to halt further oxidation of samples. In addition, 0.2% Triton X-100 detergent was added to disperse the lipids in solution.

2.2. Multilamellar vesicle preparation for lipid peroxidation

Phospholipid (PL) and cholesterol at fixed mole ratios ranging from 0.0–0.8 cholesterol-to-phospholipid (C/P), were combined with vehicle (ethanol) or drug in glass test tubes and shell-dried under a stream of N_2 gas while vortex mixing. Samples were then further dried under vacuum for 1 h to remove residual solvent. Multilamellar vesicles (MLVs) were formed by suspending the dried lipid samples in diffraction buffer at the desired concentrations of glucose or mannitol (0, 5, 10, 50 mM) to yield a final PL concentration of 1 mg/mL. Samples were then vortexed vigorously for 3 min at room temperature [17].

2.3. Multilamellar vesicle preparation for X-ray diffraction analysis

Phospholipid and cholesterol at 0.6 C/P were combined with vehicle or drug and then prepared and dried as described above. Samples were suspended in diffraction buffer or 10 mM glucose solution to yield MLVs with a PL concentration of 2.5 mg/mL. MLVs were formed by vortex mixing for 3 min at room temperature [17]. Immediately following initial MLV preparation and at each subsequent 24 h timepoint, aliquots of each membrane sample were taken for SAXS and CHOD-spectrophotometric assay measurements in order to correlate the X-ray diffraction profiles with the lipid hydroperoxide concentration values.

2.4. Lipid hydroperoxide concentration measurement

MLV samples were subjected to time-dependent autoxidation by incubating at 37 °C in an uncovered water bath. This method of autoxidation allows lipid peroxidation to occur gradually without the use of added initiators. Aliquots of each sample were removed at 24 h intervals and combined with 1 mL of active CHOD-iodide reagent. The aliquots were reduced with each successive timepoint from 100 to 10 μg of lipid to ensure that spectrophotometric absorption readings were within the optimal range. The samples were then covered and stored in darkness for at least 3 h. Triiodide (I_3^-), formed by the oxidation of iodide (I^-) by hydroperoxides, is directly proportional to the concentration of lipid hydroperoxides [16]. Through the CHOD colorimetric assay, the extent of peroxidation was determined by measuring the absorption of I_3^- (molar absorptivity coefficient (ε) of 2.46×10^4 M^{-1} cm^{-1}) at 365 nm in a Beckman Coulter DU-640 spectrophotometer (Fullerton, CA). LOOH concentration values were expressed as mean ± S.D. ($n=5$).

2.5. Preparation of samples for X-ray diffraction analysis

The effects of glucose and mannitol on membrane structure were measured over 48 h at specific timepoints. Membrane samples were oriented for X-ray diffraction as described previously [18]. Briefly, aliquots of MLVs containing 250 μg of phospholipid were transferred to Lucite sedimentation cells with preloaded aluminum foil substrates for collection of single membrane pellets. The sedimentation cells contained 0.35 μM BHT to prevent further oxidation during processing. Samples were then centrifuged in a Sorvall AH-629 swinging bucket ultracentrifuge rotor (DuPont) at 35,000 *g*, 5 °C. Samples taken at the experiment initiation and at 9 and 24 h were centrifuged for 1.5–2 h; samples analyzed beyond the 24 h period were centrifuged for 20 h. Following centrifugation, the supernatant was removed and the aluminum foil substrate, supporting the oriented membrane pellet, was mounted onto a curved glass slide. The samples were placed into either hermetically sealed brass diffraction canisters for analysis or glass vials for storage, which contained potassium tartrate ($K_2C_4H_4O_6\cdot1/2\ H_2O$) to establish a 74% relative humidity (RH) level; samples were incubated at this RH and 20 °C for at least 1 h prior to and during X-ray diffraction.

2.6. Small angle X-ray diffraction analysis

A description of SAXS theory and data analysis has been described previously [19]. Briefly, X-ray diffraction experiments were conducted by aligning the curved substrates supporting the membrane samples at grazing incidence with respect to a collimated, monochromatic CuK_α X-ray beam ($K_{\alpha1}$ and $K_{\alpha2}$ unresolved; $\lambda=1.54$ Å) produced by a Rigaku Rotaflex RU-200, high-brilliance microfocus generator (Rigaku-MSC, The Woodlands, TX). Diffraction data were collected on a one-dimensional, position-sensitive electron detector (Hecus X-ray Systems, Graz, Austria) located 150 mm from the sample. A measurement of crystalline cholesterol monohydrate was used to verify the detector calibration. The unit cell periodicity (*d*-space) of the membrane lipid bilayer was calculated as defined by Bragg's Law.

2.7. Statistical analysis

Peroxidation data are presented as mean ± S.D. Statistical differences between results from independent experimental conditions were tested using the two-tailed unpaired Student's *t*-test or one-way analysis of variance with the Dunnett multiple comparisons test. A value of $p<0.05$ was considered significant.

3. Results

3.1. Effect of glucose on lipid peroxidation

We measured the dose-dependent effects of glucose on LOOH formation in lipid vesicles composed of phospholipid with fixed amounts of PUFAs and cholesterol. The addition of 5, 10 and 50 mM glucose to the DLPC membrane vesicles at 0.6 C/P caused a significant and concentration-dependent increase in peroxidation relative to control (Fig. 1). Similar effects of glucose were observed in DAPC membrane vesicles at 0.2 C/P (data not shown). We also measured the lipid peroxidation effects of mannitol, a non-reducing sugar alcohol commonly used as a control in comparison with glucose for in vivo studies [13,20]. As shown in Fig. 1, glucose significantly amplified lipid peroxidation compared to mannitol at equivalent concentrations.

The membrane cholesterol content can also affect the efficiency of free radical propagation through a lipid bilayer by altering the physical packing of the phospholipids [15]. To examine the influence of glucose on this effect, we measured LOOH formation in DLPC membranes prepared with a range of C/P mole ratios in the absence (control) or presence of 50 mM glucose. The control samples showed no signi-

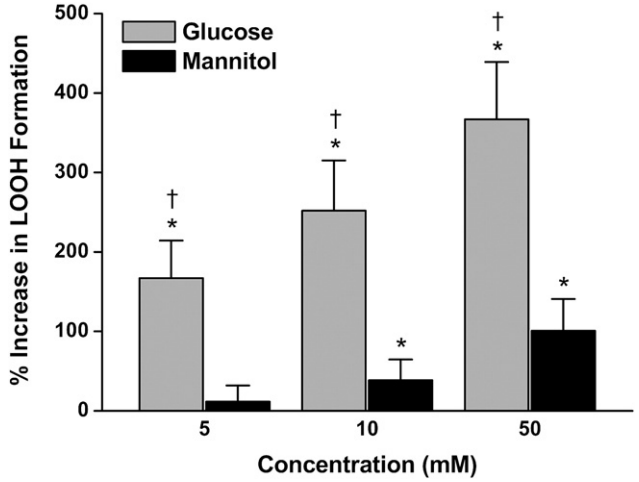

Fig. 1. Glucose promoted a dose-dependent increase in LOOH formation over mannitol in DLPC membranes. Membranes underwent autoxidation while incubated in a phosphate buffer solution containing 5, 10 or 50 mM glucose or mannitol at 37 °C for 48 h. Total levels of LOOH were measured by the CHOD-iodide assay and percent increase was calculated by normalizing to untreated control. *$p<0.01$ *versus* control (Dunnett multiple comparisons test; overall ANOVA $p<0.0001$; $F=183.86$); †$p<0.0001$ glucose *versus* equivalent mannitol concentration (Student's *t*-test); $n=5$.

ficant relationship between C/P ratios after the 48 h timepoint (data not shown); however, the addition of glucose accelerated LOOH formation and accentuated the cholesterol-dependent effect within 48 h (Fig. 2).

3.2. Membrane structural effects of glucose

Progressive lipid peroxidation has been shown to irreversibly alter the structure of biological membranes by promoting cholesterol crystalline domains and the eventual degradation of the overall phospholipid bilayer structure [15]. We measured the effect of a 10 mM glucose (the physiological equivalent of high postprandial blood sugar levels) or mannitol solution on model membrane phospholipid structure using SAXS. The diffraction data were correlated with lipid hydroperoxide values using the CHOD-iodide assay of the same samples. Immediately following preparation of membrane samples, prior to exposure to autoxidative conditions, no significant differences in membrane structure were observed for untreated, mannitol- or glucose-treated membranes (Fig. 3). The average membrane periodicity (*d*-space) was approximately 53 Å for control, mannitol- and glucose-treated samples and no cholesterol domains were detected. However, cholesterol domains were detected as early as 24 h in the glucose-treated samples (data not shown). The control and mannitol-treated samples did not begin to show these membrane changes until 48 h with no or little reduction in bilayer width. In contrast, by 48 h, the X-ray diffraction data of the glucose-treated samples showed signs of advanced membrane degradation, evidenced by a decrease in *d*-space to 49 Å, the presence of large cholesterol domain peaks and the loss of higher order phospholipid peaks (Fig. 3). These structural changes correlated with a two-fold increase in lipid hydroperoxide levels in the glucose-treated samples *versus* the mannitol-treated or untreated control samples.

3.3. Amlodipine/atorvastatin metabolite combination inhibits hyperglycemic lipid peroxidation in MLVs

Treatment of DLPC model membranes at 0.6 C/P with AML and ATM significantly attenuated the effects of 50 mM glucose (Fig. 4). LOOH formation was dramatically reduced by the combination treatment in a dose-dependent manner over a range of concentrations that correspond to pharmacologic doses (0.5 to 2.0 μM). In addition to their potent antioxidant effects, both of these drugs have physicochemical properties that allow them to partition into the membrane at the lipid/water interface [21]. These properties diminish glucose oxidative damage by scavenging the lipid hydroperoxide radicals before they can propagate and disrupt the membrane bilayer structure.

4. Discussion

It is generally accepted that there may be several reaction mechanisms responsible for the various glycoxidation and lipoxidation products resulting from the reaction of saccharides with proteins or lipids [4,6]. Past studies typically focused on the chemistry involving sugar-amine adducts and their downstream damage but this study investigated the direct physical and chemical effects of glucose on the oxidation of PUFA membranes with high cholesterol content. The results of this study demonstrate that glucose autoxidation accelerated lipid peroxidation and the subsequent oxidative changes in membrane structure and organization. Glucose, a reducing saccharide, is susceptible to reaction at its anomeric carbon with singlet oxygen or other radical initiators [22]. This redox reaction can produce a glucose radical or other reactive oxygen species (ROS), generating a pro-oxidant effect in the lipid membrane. To confirm this hypothesis, we compared glucose to mannitol, which is structurally similar and commonly used as an osmotic control in physiologic studies [20]. We showed that glucose was far more reactive than mannitol in producing lipid hydroperoxides and inducing cholesterol crystalline domain formation in DLPC membranes. The similar diffraction profiles, *d*-space values and electron density profiles (not shown) obtained for control, mannitol- and glucose-treated samples at 0 h indicate that these disparate effects were not due to differences in hydration or other membrane structural perturbations.

The high cholesterol levels used in our study also contributed to physical alterations of the membrane structure, allowing more efficient radical penetration and propagation through the bilayer. Specifically, the highly planar steroid nucleus of cholesterol reduces the intermolecular distance between adjacent PUFA chains of the lipids, thereby facilitating the rapid movement of free radicals among the acyl chains [15]. This effect is verified by the cholesterol-dependent increase in LOOH formation, which was enhanced by glucose treatment. The correlation of cholesterol domain formation with increased lipid hydroperoxide generation shown in our study

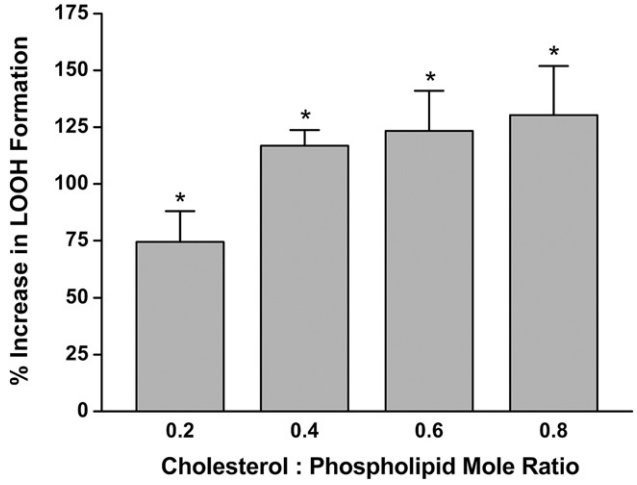

Fig. 2. Glucose accelerated cholesterol-dependent effects on LOOH formation in model membranes. DLPC membranes were incubated at 37 °C for 48 h in the presence or absence of 50 mM glucose at incremental C/P ratios. Total levels of LOOH were measured by the CHOD-iodide assay and the percent increase in LOOH was determined from comparison to untreated controls at same C/P ratio. *$p<0.001$ glucose *versus* cognate control (Student's *t*-test); $n=5$.

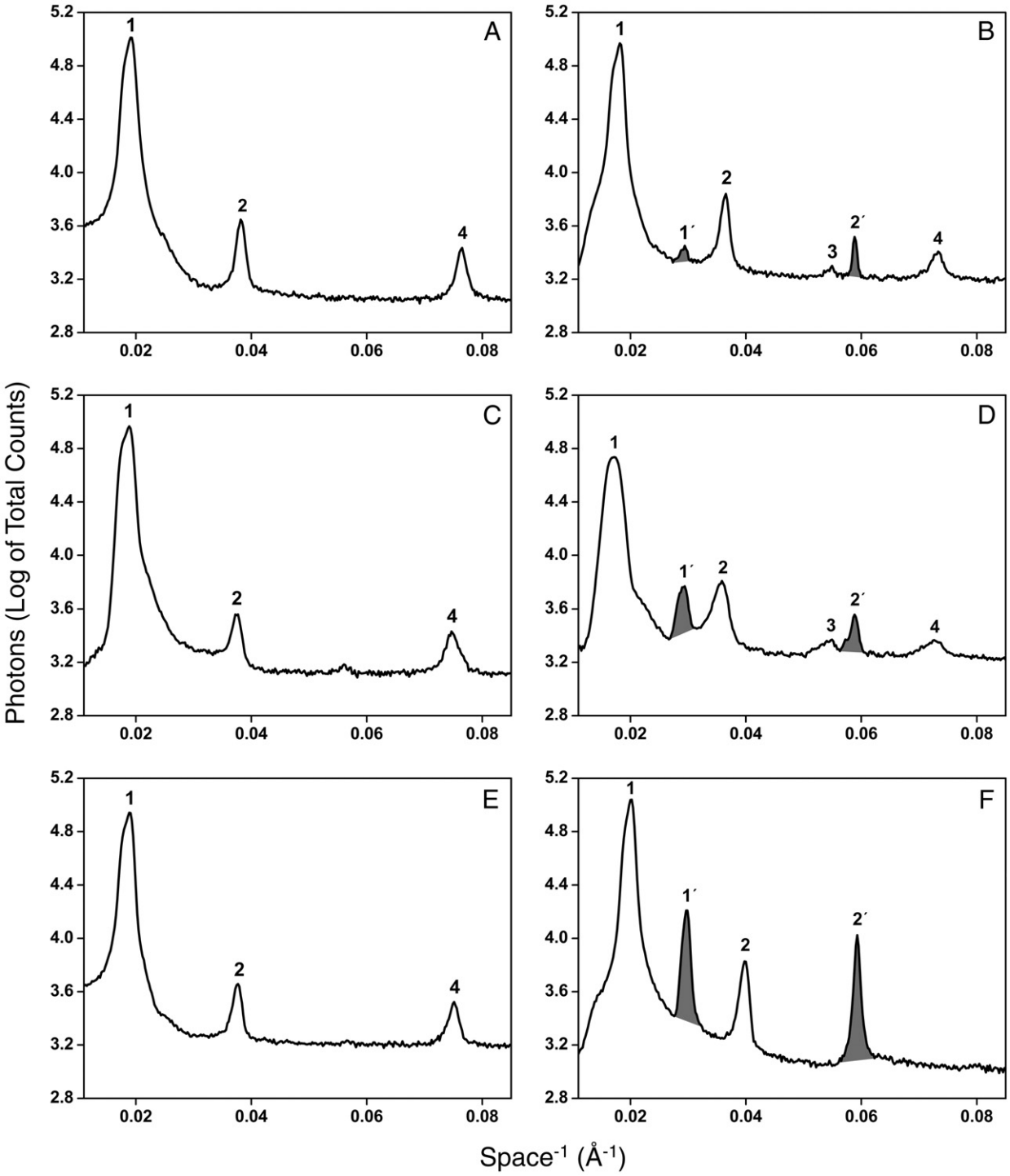

Fig. 3. Membrane lipid peroxidation and cholesterol domain formation increased with glucose treatment following 48 h of autoxidation. Small angle X-ray diffraction patterns were obtained from oriented DLPC membranes (prepared at 0.6 C/P) in the absence (A) or presence of mannitol (C) or glucose (E) at 0 h peroxidation. All three samples exhibited a single lipid phase with a *d*-space value of 53 Å. After 48 h exposure to lipid peroxidation, small cholesterol domain peaks (labeled 1′ and 2′) were observed in control (B) and mannitol-treated (D) samples. In contrast, large cholesterol domain peaks and a decrease in bilayer *d*-space (49 Å) were observed for membrane samples treated with glucose and exposed to oxidation for 48 h (F). Glucose and mannitol were tested at 10 mM in this X-ray diffraction experiment.

supports the hypothesis that hyperglycemia with hyperlipidemia may be linked to cholesterol crystal development, a hallmark feature of atherosclerotic plaque. The observed progressive formation of cholesterol crystalline domains was accompanied by a reduction in the membrane bilayer width. The reduction in width is caused by the depletion of cholesterol from within the phospholipid region into crystalline domains in addition to the physical changes of the phospholipid acyl chains caused by oxidative damage. Evidence suggests that even minor physical changes in the cell membrane may lead to the disruption of caveolae, cholesterol rich membrane domains critical to many cellular processes, leading to eNOS and insulin receptor dysfunction [12,23].

Glucose-mediated oxidative stress also contributes to the inflammatory pathways associated with diabetes and atherosclerosis pathophysiology [24]. Specifically, oxidative stress contributes to insulin resistance through an “oxidative-inflammatory cascade (OIC)”

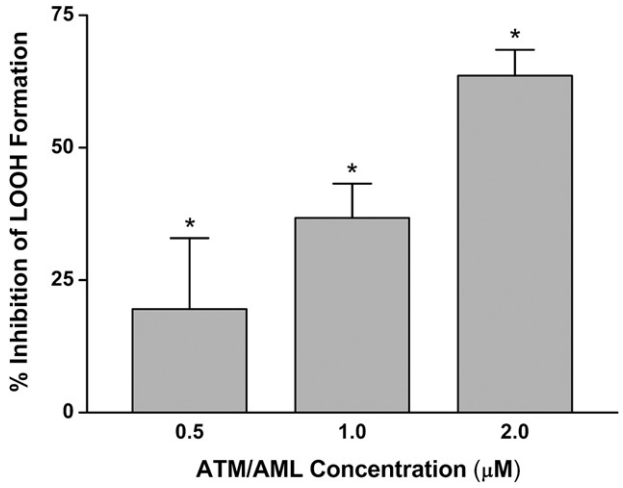

Fig. 4. Atorvastatin metabolite and amlodipine inhibited glucose oxidative effect on lipid peroxidation. DLPC membranes at 0.6 C/P were incubated with 50 mM glucose at equimolar concentrations of atorvastatin metabolite and amlodipine and underwent lipid peroxidation at 37 °C for 48 h. LOOH concentration was measured by the CHOD-iodide assay. *$p<0.01$ *versus* control without drug (Dunnett multiple comparisons test; overall ANOVA $p<0.0001$; $F=70.45$); $n=5$.

[25]. Glucose, obesity and oxidative stress reduce intracellular antioxidant defense mechanisms while activating inflammatory responses from transcription factors and kinases, such as c-Jun N-terminal kinase (JNK), protein kinase C (PKC) and inhibitor of kappa B kinase-β (IKKβ) [25,26]. Some inflammatory pathways, such as activation of IKKβ, have a causative role in the deleterious effects of high glucose on endothelial cell function [27]. Nonacetylated salicylates have been shown to block the transcription factor nuclear factor-κB (NF-κB) activity through direct inhibition of IKKβ [26,27]. A recent double-masked, placebo controlled study of 20 obese young adults found that salsalate, a dimer of salicylic acid, reduced glycemia while improving inflammatory cardiovascular risk indexes [28]. Studies with drugs possessing antioxidant activity at the cellular level, either directly or through causation, such as statins, glitazones, and angiotensin converting enzyme (ACE) inhibitors, also have shown benefit in improving insulin resistance, the OIC mechanism, and in some cases, glycemia [24,25].

Our results augment studies linking hyperglycemia and dyslipidemia to mechanisms of protein and lipid damage, LDL oxidation, eNOS dysfunction and inflammation [4,5,29]. Glycation reactions produce a cascade of imbalances disrupting normal metabolic, enzymatic and signaling pathways, which exacerbate the inflammation and vascular damage from oxidative stress and may increase insulin resistance [4,12]. Even acute periods of high glucose levels have been shown to increase oxidative damage after ischemic stroke and cardiac events [30,31]. While the current study did not utilize metal catalysts commonly associated with glucose autoxidation, such as copper or iron, the prevalence of these catalysts in the body may accelerate the effects seen here [5]. For these reasons, therapeutic interventions of metabolic disorders and cardiovascular risks should not only treat hypertension and high cholesterol but should also increase antioxidants and control blood sugar levels. Laboratory studies have demonstrated that, in addition to its powerful cholesterol lowering effects, ATM is also a potent antioxidant [32] and the combination of AML/ATM exhibits a synergistic antioxidant effect [21,33]. This study confirms that free radical-scavenging antioxidants, such as ATM, slow the progression of oxidative damage in lipid membranes even under conditions of model hyperglycemia and hyperlipidemia. The results reported here also support clinical studies showing that the synergistic benefit of the AML/ATM combination reduces cardiovascular events in patients that have various risk factors, including diabetes, as compared to other combinations [34,35].

Despite decades of research on the role of oxidative stress in the progression of diabetes, metabolic disorders, and cardiovascular disease, there is still considerable debate over the initial cause and underlying mechanisms which lead to the progression of these diseases. The results of our findings suggest that elevated reactive saccharide levels in conjunction with elevated cholesterol levels may contribute to these oxidative stress-induced disorders. Further investigation on the effect of hyperglycemia on lipid vesicle composition and size, modeling the various types of particles and membrane bilayers in vivo, may provide additional insight into the mechanisms of these pathologies.

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References

[1] R. Stocker, J.F. Keaney Jr, Role of oxidative modifications in atherosclerosis, Physiol. Rev. 84 (2004) 1381–1478.
[2] Y. Yu, T.J. Lyons, A lethal tetrad in diabetes: hyperglycemia, dyslipidemia, oxidative stress, and endothelial dysfunction, Am. J. Med. Sci. 330 (2005) 227–232.
[3] M.E. Tushuizen, M. Diamant, R.J. Heine, Postprandial dysmetabolism and cardiovascular disease in type 2 diabetes, Postgrad. Med. J. 81 (2005) 1–6.
[4] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[5] H.M. Knott, B.E. Brown, M.J. Davies, R.T. Dean, Glycation and glycoxidation of low-density lipoproteins by glucose and low-molecular mass aldehydes. Formation of modified and oxidized particles, Eur. J. Biochem. 270 (2003) 3572–3582.
[6] S. Pennathur, J.W. Heinecke, Mechanisms for oxidative stress in diabetic cardiovascular disease, Antioxid. Redox. Signal 9 (2007) 955–969.
[7] R. Bucala, Z. Makita, T. Koschinsky, A. Cerami, H. Vlassara, Lipid advanced glycosylation: pathway for lipid oxidation in vivo, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 6434–6438.
[8] T. Obšil, E. Amler, V. Obšilova, Z. Pavlíček, Effect of aminophospholipid glycation on order parameter and hydration of phospholipid bilayer, Biophys. Chem. 80 (1999) 165–177.
[9] R. Medina-Navarro, G. Duran-Reyes, M. Diaz-Flores, J.J. Hicks, J. Kumate, Glucose-stimulated acrolein production from unsaturated fatty acids, Hum. Exp. Toxicol. 23 (2004) 101–105.
[10] S. Pennathur, Y. Ido, J.I. Heller, J. Byun, R. Danda, P. Pergola, J.R. Williamson, J.W. Heinecke, Reactive carbonyls and polyunsaturated fatty acids produce a hydroxyl radical-like species: a potential pathway for oxidative damage of retinal proteins in diabetes, J. Biol. Chem. 280 (2005) 22706–22714.
[11] J.A. Berliner, A.D. Watson, A role for oxidized phospholipids in atherosclerosis, N. Engl. J. Med. 353 (2005) 9–11.
[12] M. Bertelsen, E.E. Änggård, M.J. Carrier, Oxidative stress impairs insulin internalization in endothelial cells in vitro, Diabetologia 44 (2001) 605–613.
[13] N. Lamharzi, C.B. Renard, F. Kramer, S. Pennathur, J.W. Heinecke, A. Chait, K.E. Bornfeldt, Hyperlipidemia in concert with hyperglycemia stimulates the proliferation of macrophages in atherosclerotic lesions: potential role of glucose-oxidized LDL, Diabetes 53 (2004) 3217–3225.
[14] R.P. Mason, T.N. Tulenko, R.F. Jacob, Direct evidence for cholesterol crystalline domains in biological membranes: role in human pathobiology, Biochim. Biophys. Acta 1610 (2003) 198–207.
[15] R.F. Jacob, R.P. Mason, Lipid peroxidation induces cholesterol domain formation in model membranes, J. Biol. Chem. 280 (2005) 39380–39387.
[16] M. El-Saadani, H. Esterbauer, M. El-Sayed, M. Goher, A.Y. Nassar, G. Jurgens, A spectrophotometric assay for lipid peroxides in serum lipoproteins using commercially available reagent, J. Lipid Res. 30 (1989) 627–630.
[17] A.D. Bangham, M.M. Standish, J.C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 13 (1965) 238–252.
[18] L. Herbette, J. Marquardt, A. Scarpa, J.K. Blasie, A direct analysis of lamellar X-ray diffraction from hydrated oriented multilayers of fully functional sarcoplasmic reticulum, Biophys. J. 20 (1977) 245–272.
[19] R.P. Mason, G.E. Gonye, D.W. Chester, L.G. Herbette, Partitioning and location of Bay K 8644, 1,4-dihydropyridine calcium channel agonist, in model and biological membranes, Biophys. J. 55 (1989) 769–778.
[20] S.K. Jain, K.M. Morshed, K. Kannan, K.E. McMartin, J.A. Bocchini Jr, Effect of elevated glucose concentrations on cellular lipid peroxidation and growth of cultured human kidney proximal tubule cells, Mol. Cell. Biochem. 162 (1996) 11–16.
[21] R.P. Mason, M.F. Walter, C.A. Day, R.F. Jacob, Intermolecular differences of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors contribute to distinct pharmacologic and pleiotropic actions, Am. J. Cardiol. 96 (2005) 11F–23F.

[22] V. Jakus, N. Rietbrock, Advanced glycation end-products and the progress of diabetic vascular complications, Physiol. Res. 53 (2004) 131–142.
[23] A.W. Cohen, T.P. Combs, P.E. Scherer, M.P. Lisanti, Role of caveolin and caveolae in insulin signaling and diabetes, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E1151–1160.
[24] A. Ceriello, E. Motz, Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 816–823.
[25] R.E. Lamb, B.J. Goldstein, Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function, Int. J. Clin. Pract. 62 (2008) 1087–1095.
[26] H. Tilg, A.R. Moschen, Inflammatory mechanisms in the regulation of insulin resistance, Mol. Med. 14 (2008) 222–231.
[27] F. Kim, K.A. Tysseling, J. Rice, B. Gallis, L. Haji, C.M. Giachelli, E.W. Raines, M.A. Corson, M.W. Schwartz, Activation of IKKbeta by glucose is necessary and sufficient to impair insulin signaling and nitric oxide production in endothelial cells. J. Mol. Cell. Cardiol. 39 (2005) 327–334.
[28] A. Fleischman, S.E. Shoelson, R. Bernier, A.B. Goldfine, Salsalate improves glycemia and inflammatory parameters in obese young adults. Diabetes Care 31 (2008) 289–294.
[29] K. Posch, S. Simecek, T.C. Wascher, G. Jurgens, S. Baumgartner-Parzer, G.M. Kostner, W.F. Graier, Glycated low-density lipoprotein attenuates shear stress-induced nitric oxide synthesis by inhibition of shear stress-activated L-arginine uptake in endothelial cells, Diabetes 48 (1999) 1331–1337.
[30] R. Garg, A. Chaudhuri, F. Munschauer, P. Dandona, Hyperglycemia, insulin, and acute ischemic stroke: a mechanistic justification for a trial of insulin infusion therapy, Stroke 37 (2006) 267–273.
[31] P. Dandona, A. Chaudhuri, H. Ghanim, P. Mohanty, Proinflammatory effects of glucose and anti-inflammatory effect of insulin: relevance to cardiovascular disease, Am. J. Cardiol. 99 (2007) 15B–26B.
[32] R.P. Mason, M.F. Walter, C.A. Day, R.F. Jacob, Active metabolite of atorvastatin inhibits membrane cholesterol domain formation by an antioxidant mechanism, J. Biol. Chem. 281 (2006) 9337–9345.
[33] R.P. Mason, A rationale for combination therapy in risk factor management: a mechanistic perspective, Am. J. Med. 118 (Suppl 12A) (2005) 54–61.
[34] R. Fogari, P. Preti, A. Zoppi, P. Lazzari, L. Corradi, E. Fogari, L. Ciccarelli, G. Derosa, Effects of amlodipine-atorvastatin combination on inflammation markers and insulin sensitivity in normocholesterolemic obese hypertensive patients, Eur. J. Clin. Pharmacol. 62 (2006) 817–822.
[35] P. Sever, B. Dahlof, N. Poulter, H. Wedel, G. Beevers, M. Caulfield, R. Collins, S. Kjeldsen, A. Kristinsson, G. McInnes, J. Mehlsen, M. Nieminem, E. O'Brien, J. Ostergren, Potential synergy between lipid-lowering and blood-pressure-lowering in the Anglo-Scandinavian Cardiac Outcomes Trial, Eur. Heart J. 27 (2006) 2982–2988.